PEG Thiol Reagents

Introduction

Thiol PEG is a thiol-terminated polyethylene glycol (PEG)-containing reagent with either a methyl ether, hydroxyl or carboxylic acid group at the other end. These reagents have defined molecular weights and spacer lengths and are used for modifying surfaces such quantum dots, self-assembled monolayers and magnetic particles. Functionalization of solid surfaces with PEG spacers significantly reduces nonspecific protein binding in the application.

- The Thiol PEG reagents are low-melting solids or liquid that are difficult to weigh and dispense. To facilitate handling, make a stock solution by dissolving the reagent in dimethylsulfoxide (DMSO) or dimethylformamide (DMF).
- Store unused stock solution at -20°C. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation. To minimize air exposure, keep the stock solution under an inert gas such as argon or nitrogen. Cap the stock solution with a septum and use a syringe to remove the solution.
- If the Carboxyl-Thiol PEG reagent is used for surface binding and further protein loading, the reagent-to- surface ratio in the reaction affect the number of carboxylic acid residues available for further modification. Optimize these ratios to obtain the modification level needed for the specific application.
- Use non-amine-containing buffers at pH 7-9 such as PBS (20mM sodium phosphate, 150mM NaCl; pH 7.4); 100mM carbonate/biocarbonate; or 50mM borate. Do not use buffers that contain primary amines, such as Tris or glycine, which compete with acylation.

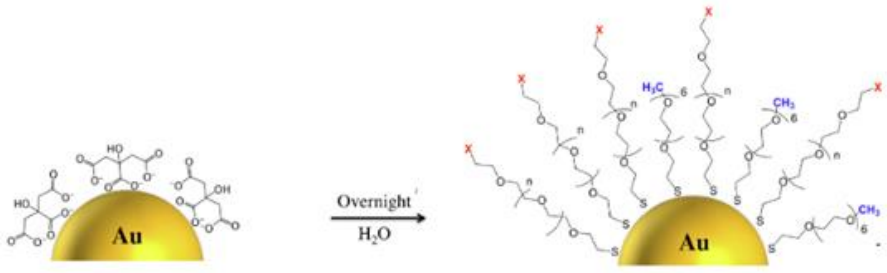

Figure 1. Scheme of the NP modification with PEG and antibody (as passivating agent).

Application

Gold nanoparticles synthesis

In a three-neck round-bottom flask containing a stir bar, 1.4 mL of trisodium citrate (0.15 M) were injected into 50 mL of boiling aqueous potassium tetrachloroaurate (3 mM) at pH 7. After addition, the reaction mixture changed from yellow, to colorless, to black and finally to purple-red within 1 min. The solution was refluxed for five minutes and then quenched to room temperature. The freshly synthesized GNP suspension was then dialyzed against a 1 mM citrate solution during 12 h. Images of the GNPs were obtained with a Philips CM20-UltraTWIN Transmission Electron Microscope (TEM) equipped with a lanthanum hexaboride (LaB6) crystal at a 200 kV accelerating voltage. The average size and standard deviation were determined by measuring the size of more than 100 GNPs. The GNP concentration was determined by UV–Vis absorption spectroscopy using the extinction coefficient calculated by making the assumption that all the gold ions added during the

synthesis have been reduced into nanoparticles (the remaining gold salts were below detection limit of ICP) and knowing the GNPs size. As an example, for GNPs of 16 nm, the GNP concentration of the solution was estimated to be around 23 nM and their extinction coefficient to be 4.3×10^8 M^{-1} cm^{-1}.

GNPs functionalization

Note: Other thiol-PEG-X (X: COOH, NH2, alkyne, OMe, etc) also can be used for this application.

An excess of HS-PEGs was added to citrate-capped aqueous GNPs suspensions (>3 $\times 10^4$ HS-PEGs/GNP) and the resulting suspensions were stirred overnight. The particles were subsequently cleaned by centrifugation (18 min at 17,000g) and replacement of the supernatant by an equal volume of water. This process was performed 6 times, which corresponds to a dilution of the soluble species (mostly HS-PEGs and citrate) by a factor of 5×10^6.

Conjugation

Note: the procedure below is for carboxyl-PEG-SH modified nanoparticle, for other functional groups modified nanoparticle, check their application note.

1. The newly introduced carboxylic acid groups can be activated by adding appropriate amounts of EDC and NHS to the modified surface in MES-buffered saline (0.1M MES, 0.5M NaCl; pH 6.0 or 0.1M MES, 0.9% NaCl; pH 4.7) and reacting for 15 minutes at room temperature.
2. Wash the surface with MES-buffered saline to remove any remaining EDC and NHS.
3. Add the desired amine-containing substrate (prepared in PBS buffer) to the activated surface and react for 2 hours at room temperature.
4. Add hydroxylamine or another amine-containing buffer to quench the conjugation reaction.